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Role of Plasma Lipoproteins in Modifying the Toxic Effects of Water-Insoluble Drugs: Studies With Cyclosporine A

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ABSTRACT Lipoproteins are a heterogeneous population of macromolecular aggregates of lipids and proteins that are responsible for the transport of lipids through the vascular and extravascular fluids from their site of synthesis or absorption to peripheral tissues. Lipoproteins are involved in other biological processes as well, including coagulation and tissue repair, and serve as carriers of a number of hydrophobic compounds within the systemic circulation. It has been well documented that disease states (eg, AIDS, diabetes, cancer) significantly influence circulating lipoprotein content and composition. Therefore, it appears possible that changes in the lipoprotein profile would affect not only the ability of a compound to associate with lipoproteins but also the distribution of the compound within the lipoprotein subclasses. Such an effect could alter the pharmacokinetics and pharmacological action of the drug. This paper reviews the factors that influence the interaction of one model hydrophobic compound, cyclosporine A, with lipoproteins and the implications of altered plasma lipoprotein concentrations on the pharmacological behavior of this compound.

INTRODUCTION Lipoproteins are a heterogeneous population of macromolecular aggregates of lipids and proteins that are responsible for the transport of lipids through the vascular and extravascular fluids from their site of synthesis or absorption to peripheral tissues [1, 2]. Lipids, which include Triglycerides (TG) and cholesteryl esters (CE), are delivered from the liver and intestine to other tissues for storage or catabolism in the production of energy. Lipoproteins are involved in other biological processes as well, including coagulation and tissue repair, and serve as carriers of a number of hydrophobic compounds within the systemic circulation [1, 2].

It has been well documented that disease states significantly influence circulating lipoprotein content and composition. Therefore, it appears possible that changes in the lipoprotein profile would affect not only the ability of a compound to associate with lipoproteins but also the distribution of the compound within the lipoprotein subclasses. Such an effect could alter the pharmacokinetics and pharmacological action of the drug.

This paper reviews the factors that influence the interaction of one model hydrophobic compound, cyclosporine A (CSA), with lipoproteins and the implications of altered plasma lipoprotein concentrations on the pharmacological behavior of this compound.

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LIPOPROTEIN STRUCTURE Lipoproteins are spherical particles consisting of a nonpolar lipid core (TG and CE) surrounded by a surface monolayer of amphipathic lipids (phospholipids and unesterified cholesterol) and specific proteins called apolipoproteins (1,2. A number of different phospholipids are incorporated into the coat of the lipoprotein, the more common of which are phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and sphingomyelin. The most abundant of these phospholipids is phosphatidylcholine, which is also utilized as a substrate in the esterification of cholesterol to cholesteryl ester by the enzyme lecithin:cholesterol acyltransferase. Since lipids, in general, have lower buoyant densities than proteins, lipoproteins with a larger amount of lipid relative to protein will have a lower density than lipoproteins with a smaller lipid-to-protein ratio [1,2]. Traditionally, plasma lipoproteins are classified and separated according to their density and are divided into 5 main categories: chylomicrons, very low density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) [1,2].

Chylomicrons, with a diameter of approximately 100 to 1000 nm, are the largest of the lipoproteins and are found in greatest abundance after a meal. Chylomicrons are synthesized by the intestine and are core-rich in TG derived from dietary fat. VLDL are the next largest lipoproteins (diameter = 30-80 nm) and are also rich in TG. VLDL are synthesized mainly by the liver but may also be synthesized to a lesser degree by the intestine.

IDL, whose lipid core is composed mainly of CE with some TG, are the product of VLDL metabolism. LDL, in turn, are the product of IDL metabolism in which nearly all of the remaining TG have been hydrolyzed to produce a lipoprotein core composed almost entirely of CE. LDL are the major cholesterol-carrying lipoproteins and are the second smallest of the lipoproteins, with an average diameter of 18 to 25 nm. The smallest of the lipoproteins, with a diameter of approximately 7 to 12 nm, are HDL. These lipoproteins are diverse in both structure and function and have a lipid core that contains both CE and TG of varying ratios.

CSA AND ITS INTERACTION WITH PLASMA LIPOPROTEINS CSA is a cyclic polypeptide of fungal origin discovered in the early 1970s and approved for use in 1983 [3, 4]. It interacts with the intracellular protein cyclophilin, which inhibits the calcium-dependent translocation of nuclear transcription factors, which are necessary for interleukin-2 gene transcription. Since interleukin-2 is required for the proliferation of T lymphocytes, CSA is capable of diminishing the immune response [3, 4]. However, despite its effectiveness as an immunosuppressant, the use of CSA is limited by renal toxicity, which is characterized by a rise in serum creatinine and a decrease in the glomerular filtration rate [3].

A number of laboratories, including ours, have shown CSA to associate with lipoproteins upon incubation in human plasma [2, 4-10], resulting in a modification of its pharmacological activity. De Kippel et al [6] have reported decreases in CSA activity in patients who have elevated plasma triglyceride levels, while de Groen et al [7] have observed increases in cyclosporine toxicity in those with hypolipidemia. Lemaire et al have observed enhanced antiproliferative effects of CSA when the drug was associated with LDL; these effects were not evident when the drug was associated with either VLDL or HDL [9, 10]. Gardier et al observed that heart transplant patients with high total plasma cholesterol levels demonstrated increased CSA association with plasma LDL and increased CSA-induced renal toxicity compared with normolipidemic controls [8]. In addition, Arnadottir et al observed elevations in CSA-induced renal toxicity in kidney transplant patients who exhibited increases in plasma cholesterol concentration [11]. Taken together, these studies provide substantial evidence suggesting that plasma lipoprotein lipid levels have a major impact on the efficacy and toxicity of CSA.

FACTORS THAT INFLUENCE CSA INTERACTIONS WITH PLASMA LIPOPROTEINS The association of CSA with plasma lipoproteins appears to significantly influence the drug's efficacy and safety, particularly since it is often administered to patients who have abnormal lipid metabolism (ie, hypo/hypercholesterolemia and/or hypertriglyceridemia). Our laboratory has investigated 2 different characteristics of dyslipidemic plasma that may influence the association of CSA with plasma lipoproteins and thus modify its pharmacokinetics and pharmacodynamics. These 2 characteristics are [1] changes in the rate of transfer of esterified cholesterol (CE) and TG between different lipoprotein classes, and [2] changes in plasma lipoprotein lipid and protein content.

INFLUENCE OF CHOLESTERYL ESTER TRANSFER PROTEIN Cholesteryl ester transfer protein (CETP) is a 476-amino-acid hydrophobic glycoprotein with a molecular weight of 74 kD [12, 13]. CETP, along with phospholipid transfer protein (PLTP), is believed to have evolved from a common ancestor belonging to the family of lipopolysaccharide binding proteins [14]. The 2 proteins share 20% homology and have homologous regions to lipopolysaccharide binding proteins [14]. CETP expression between mammalian species is variable, with undetectable levels in rats and mice and moderate levels in humans and rabbits [15]. The majority of CETP in humans is synthesized in the liver, with lower levels produced in the adipose tissue, kidney, heart, and spleen [16, 17].

CETP facilitates the transfer of CE from HDL to apoB-containing lipoproteins (VLDL and LDL) with a reciprocal transfer of TG [16, 18-20]. CETP, along with PLTP, plays an important role in the metabolism and remodeling of plasma lipoproteins [21, 22]. CETP may also play a role in certain disease processes such as atherosclerosis by redistributing cholesterol from the antiatherogenic HDL particles to the proatherogenic LDL particles. Conversely, CETP is also implicated in the process of reverse cholesterol transport, which removes cholesterol from peripheral tissues and is viewed as antiatherogenic. The exact role of CETP in the development of atherosclerosis remains uncertain and is the subject of various studies [17].

Since the human body may recognize hydrophobic compounds as lipidlike particles, we have hypothesized that an increase in CETP concentration may facilitate the movement of drugs such as CSA among different lipoprotein classes. Evidence to support this hypothesis is presented in the following paragraphs.

When CSA was incubated in human plasma with or without additional supplementation of CETP, for 60 minutes at 37ºC, increases in CETP concentration resulted in an increased percentage of CSA recovered in the HDL/lipoprotein-deficient plasma (LPDP) fraction (**Table 1**) [23].

Additional experiments—designed to directly measure the potential role of CETP to facilitate CSA transfer—demonstrated that CETP-mediated percentage transfer of CE among HDL and LDL particles in human plasma was significantly different from that of CSA (**Table 2**) [23]. The differences in the percentage transfer of CE versus CSA may be attributed to an ability of CETP to transfer

lipids and drugs separately. Furthermore, differences could be attributed to the ability of HDL and LDL particles to accumulate a higher amount of CE than CSA.

In addition, when CETP-mediated transfer of CE between HDL and LDL was inhibited by TP2 (a monoclonal antibody directed against CETP), only the transfer of CSA from LDL to HDL was significantly decreased (**Table 2**) [23]. These results suggest that the transfer of CSA from LDL to HDL is partially facilitated by CETP, while the transfer of CSA from HDL to LDL is facilitated not by CETP but by other plasma factors and/or by spontaneous transfer. This notion is supported by the work of Hughes et al, who hypothesize that the plasma distribution of CSA is determined by factors other than simple diffusion between lipoprotein particles [24].

These findings suggest that the distribution/redistribution of CSA among plasma lipoproteins facilitated by CETP may serve as a possible mechanism for determining the ultimate fate of these compounds.

INFLUENCE OF LIPOPROTEIN CONCENTRATION AND COMPOSITION A second feature of dyslipidemic plasma is the increase and/or decrease in plasma lipoprotein cholesterol and triglyceride concentrations. Since CSA associates with plasma lipoproteins following their administration, our laboratory has hypothesized that changes in lipoprotein concentration and composition would alter the lipoprotein distribution of CSA.

Our laboratory reported that broad plasma dyslipidemias (**Table 3**) and specific increases in LDL and VLDL lipid levels (**Table 4**) resulted in an increasing amount of CSA recovered in these fractions and a significant decrease in the amount of CSA recovered in the HDL fraction (Tables 3 and 4) [5]. Furthermore, the amount of drug recovered in the nonlipoprotein fraction remained unchanged. These findings suggest that CSA lipoprotein distribution may be partially regulated by plasma lipoprotein cholesterol and to a lesser extent triglyceride concentrations. It further suggests that the redistribution of drug from one lipoprotein class (HDL) to another (LDL or VLDL) could be influenced by different disease states and adjunct therapies such as intralipid infusion, in which lipoprotein plasma concentrations and composition are altered [25].

In addition, we observed that increasing the TG:total cholesterol (TC) ratio within VLDL and HDL resulted in more CSA recovered in the VLDL fraction but less CSA recovered in the HDL fraction [5]. However, increases in the HDL TG:TC ratio increased the amount of drug recovered in the HDL fraction [5]. These findings suggest that not only lipid mass (TC and TG) and lipoprotein composition but also the type of lipoprotein in which these changes occur is another possible factor in determining the lipoprotein CSA associates with. Since transplantation patients exhibit lipid disturbances, including decreased cholesterol levels and/or elevated triglyceride levels, these results may provide an explanation for the unpredictable and inconsistent pharmacokinetics and pharmacodynamics of CSA following administration.

CELLULAR UPTAKE AND TOXICITY STUDIES It has been suggested that cellular uptake of CSA may be mediated through hepatic HDL [24] and LDL receptors [26], although others have reported that lipoproteins may not serve as vehicles for cellular uptake of CSA into hepatic-derived cells [27]. It has been suggested that CSA availability to tissue, and hence its pharmacological or toxic effects, may depend on the lipoprotein with which CSA is associated [9]. An enhanced antiproliferative effect of CSA has been observed when the drug was associated with LDL but not with VLDL or HDL [10].

Recently, we have shown that the uptake and toxicity of CSA within LLC-PK1 pig kidney cells were effectively reduced with elevated LDL concentrations (**Figure 1**) but showed a significant increase when incubated with elevated concentrations of apoA-I (data not shown) [28]. Increasing VLDL and HDL concentrations slightly reduced CSA toxicity and uptake but showed little effect with increased incubation time. Triglyceride and cholesterol, the respective major components of VLDL and LDL, did not alter CSA uptake or toxicity under the conditions tested. LDL and apoA-I are identified as the major effectors of CSA toxicity and uptake in LLC-PK1 cells. The data presented here clearly demonstrate a relationship between CSA-induced toxicity and the nature of the associated lipoprotein [28]. Taken together, these findings suggest that CSA uptake may be mediated through receptors such as the LDL receptor or those involved in protein reabsorption.

Table 1. Effect of CETP on the Distribution of CSA Into Plasma Lipoproteins After 60 Minutes of Incubation in Pooled Human Plasma*

Amount of CETP† (μg protein)	HDL/LPDP (%)‡	LDL/VLDL (%)‡
0	51 +/− 1	49 +/− 5
0.5	57 +/− 4§	43 +/− 4
1.0	59 +/− 3§	40 +/− 1§
2.0	61 +/− 1§	38 +/− 1§

*Adapted from Wasan et al.[23] Data were expressed as mean +/− standard deviation (n = 6). CETP indicates cholesteryl ester transfer protein; CSA, cyclosporine A; HDL, high-density lipoproteins; LPDP, lipoprotein-deficient plasma; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
†Amount of exogenous CETP added to 1 mL of human plasma. Endogenous CETP concentration was 1 μg of protein/mL for all test samples.
‡Percentage of initial CSA incubated in human plasma. Total recovery >98%.
§P<.05 versus HDL/LPDP or LDL/VLDL fraction at CETP = 0.

Table 2. Percent Transfer of CE and CSA from HDL to LDL and LDL to HDL, in the Presence or Absence of a Monoclonal Antibody (TP2) Directed Against CETP in Human Plasma*

Treatment	HDL to LDL (% kt)†		LDL to HDL (% kt)†	
	CE	CSA	CE	CSA
Without TP2	18 +/− 3.5	8.6 +/− 1.4	12.5 +/− 1.8	19.8 +/− 6.1
With TP2	2.2 +/− 0.9‡	10.3 +/− 2.2	2.9 +/− 1.4‡	7.5 +/− 2.6‡

*Adapted from Wasan et al.[23] Data were expressed as mean +/− standard deviation (n = 6). CE indicates cholesteryl ester; CSA, cyclosporine A; HDL, high-density lipoproteins; LDL, low-density lipoproteins; CETP, cholesteryl ester transfer protein.
†Percent fraction of labeled CE and CSA transferred per unit time.
‡P<.05 versus without TP2.

Table 3. Distribution of CSA at 1000 ng/mL Within Normolipidemic and Dyslipidemic Plasma From Different Human Subjects Following Incubation for 60 Minutes at 37°C*

Plasma Type	% VLDL/LDL Fraction†	% HDL Fraction	% LPDP Fraction
Normolipidemic	31.9 +/− 3.6	44.4 +/− 4.2	19.7 +/− 3.1
Hypercholesterolemic	46.3 +/− 7.7‡	20.9 +/− 7.7‖	20.9 +/− 2.6
Hypertriglyceridemic	54.3 +/− 13.1§	20.0 +/− 4.6‖	18.9 +/− 7.3
Hypercholesterolemic + hypertriglyceridemic	55.3 +/− 9.2§	20.1 +/− 3.7‖	12.9 +/− 5.0‡

*Adapted from Wasan et al.[5] Data expressed as mean +/− standard deviation (n = 6 patients in each group). CSA indicates cyclosporine A; VLDL, very low density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; LPDP, lipoprotein-deficient plasma.

†Percentage of initial CSA concentration incubated.

‡P<.05.

§P<.01.

‖P<.001 versus normolipidemic plasma type. Plasma types: normolipidemic (total cholesterol = 100-200 mg/dL and total triglyceride = 100-200 mg/dL), hypercholesterolemic (total cholesterol = 250-300 mg/dL), hypertriglyceridemic (total triglyceride = 350-500 mg/dL), hypercholesterolemic + hypertriglyceridemic (total cholesterol = 250-300 mg/dL and total triglyceride = 350-500 mg/dL). Total CSA recovery was greater than 88%.

Table 4. Distribution of CSA at 1000 ng/mL Within Plasma From 3 Different Patients Following Incubation for 60 Minutes at 37°C*

Patient Profile	VLDL Fraction (%)†	LDL Fraction (%)	HDL Fraction (%)	LPDP Fraction (%)	TC (mg/dL)	Total Triglyceride (mg/dL)
Patient I	13 +/− 6	27 +/− 0.4	47 +/− 5	8 +/− 1	113	79
Patient II	17 +/− 6	34 +/− 5	41 +/− 8	5 +/− 1	179	167
Patient III	19 +/− 7	41 +/− 3‡	28 +/− 2‡§	7 +/− 1	235	394

*Adapted from Wasan et al.[5] Data expressed as mean +/− standard deviation (n = 6 replicates). CSA, cyclosporine A; VLDL, very low density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; LPDP, lipoprotein-deficient plasma; TC, total cholesterol; TG, total triglycerides

†Percentage of initial CSA concentration incubated.

‡P<.05 versus patient I.

§P<.05 versus patient II.

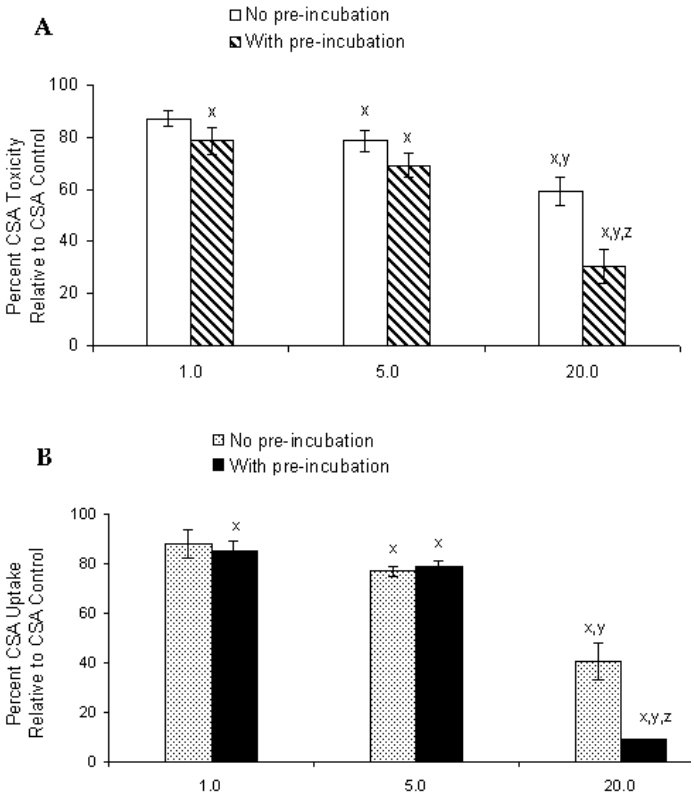

Figure 1. Effects of LDL on relative CSA toxicity (A) and uptake (B) in LLC-PK1 cells with and without preincubation of LDL. x = *P*<.05 versus CSA alone, y = *P*<.05 versus 1 ug/mL LDL in same series, z = *P*<.05 versus same LDL concentration with no preincubation. Data are presented as mean ± standard deviation, with n = 6 for all groups. Adapted from Peteherych and Wasan.[28]

CONCLUSIONS Lipoproteins are a heterogeneous population of macromolecular aggregates of lipids and proteins responsible for the transport of lipids through the vascular and extravascular fluids from their site of synthesis or absorption to peripheral tissues. Lipoproteins are involved in other biological processes as well, including coagulation and tissue repair, and serve as carriers of a number of hydrophobic compounds within the systemic circulation. It has been well documented that disease states (eg, AIDS, diabetes, cancer) significantly influence circulating lipoprotein content and composition. Therefore, it appears possible that changes in the lipoprotein profile would affect not only the ability of a compound to associate with lipoproteins but also the distribution of the compound within the lipoprotein subclasses. Such an effect could alter the pharmacokinetics and pharmacological action of the drug. This paper reviewed the factors that influence the interaction of CSA with lipoproteins and the implications of altered plasma lipoprotein concentrations on the renal cytotoxicity of this compound.

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